SOME ASPECTS OF ADRENAL ENZYME INTERRELATIONSHIPS

BY

GENE FREDERICK LATA

B.S., City College of New York, 1942

M.S., University of Illinois, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
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R. UNIV. BIBLIOTHEEK LEIDEN

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Introduction

Past studies on the adrenal gland have established the cortex of this organ as the source of a host of steriods. Of the 28 which have been identified only 6 have been shown to possess biological activity (1). Among all the cortical steroids, cholesterol is present in the adrenal cortex in the highest concentration (about 5 per cent in the guinea pig adrenal). The cholesterol itself is biologically inactive but its presence in such a high concentration implies an important function. It is known that under conditions such as shock or injection of ACTH the adrenal cholesterol is depleted, accompanied by an increased secretion of adrenal steroids (2). All this lends circumstantial evidence to the hypothesis that cholesterol is the precursor of the other steroids. *In vivo* experiments have already indicated that cholesterol can be a source compound for cholic acid (3), cholestenone (4), pregnanediol and progesterone (5). Extensive work by Chaikoff et al (6) and Rittenberg et al (7) have clearly shown that acetate is incorporated into the cholesterol molecule both *in vivo* and *in vitro*. The idea suggests itself that this latter transformation might be via the "two-carbon fragment."

Theoretical

With a view to obtaining direct evidence concerning the fate of cholesterol in the adrenal cortex, its probable role as precursor for other steroids and to test the validity of the "two-carbon fragment" route, *in vitro* enzymatic studies were made with incubating adrenal homogenates. The possibility that certain known basic enzyme systems which contribute to the "two-carbon pool," α-ketoglutaric acid dehydrogenase and fatty acid oxidase, might boost cholesterol synthesis was approached as follows: First, by some investigations into the mechanism of α-ketoglutaric acid oxidation; secondly, by a search for fatty acid oxidase in the cortex of the adrenal.

Changes in cholesterol concentrations in adrenal homogenates would be more pronounced if the initial value for cholesterol were low. To realize this situation, shocking by means of histamine injection was investigated.

It was necessary to develop an analytical scheme to detect changes in cholesterol concentrations in the incubating mixtures as well as a method for the demonstration of changes in other steroids.

Experimental

All work with the α-ketoglutaric acid oxidation was done using the constant volume Warburg apparatus for the oxygen uptake measurements and an all-glass set-up for ascending paper chromatography. Rat skeletal muscle and rat heart were used as enzyme sources.

Fatty acid oxidase activity was also studied with the Warburg apparatus.

Cholesterol was measured by the procedure developed in the present studies; in conjunction with this method the Zaffaroni technique for separation of corticosteroids by means of descending paper chromatography (8) was employed together with the Haines "Ultra-violet Scanner" (9).

Results and Discussion

1. α-Ketoglutaric Acid Oxidation—A method was devised to detect the presence of succinyl phosphate by conversion of this mixed anhydride to the hydroxamate, separation by paper chromatography and formation of a colored complex with ferric ion under acid conditions. Application of this method to α-ketoglutaric acid oxidation studies did not reveal the presence of succinyl phosphate as an intermediate under the conditions used. Other data supported the idea that cytochrome *c*, DPN and ADP were essential components of the enzyme system. Infra-red analyses indicated that in aqueous solution α-ketoglutaric acid exists mainly in a straight chain, keto-acid form. Initial phosphorylation of the molecule may therefore involve this species or a transient hydrated form.

2. Fatty Acid Oxidase—It is concluded that this enzyme is present in the adrenal gland. The activity evidenced was low, due either to improper experimental conditions or to an actually low concentration of the enzyme in the gland.

3. Histamine Shock—It was found that intraperitoneal injection of 3.0 mg of histamine per kilogram body weight will lower the adrenal cholesterol of the guinea pig by 30 per cent.

4. Procedure for Cholesterol Analysis—Cholesterol is separated from adrenal incubation mixtures by absorption of an aliquot on a cot-

ton roll, drying and extraction of the roll in a micro-Soxhlet apparatus. This extract, after treatment with alkali to hydrolyze the cholesterol esters, is eventually sampled for paper chromatography. The cholesterol is effectively separated, extracted from the paper strip in the micro-Soxhlet and analyzed by the Liebermann-Burchard reaction. The overall precision of the method is 6 per cent.

5. Other Steroids—The procedure developed for cholesterol was combined with the Zaffaroni method of separation of corticosteroids and with the use of the Haines "Ultra-violet Scanner" for following changes in steroids other than cholesterol.

6. Effect of Various Substrate Additions on the Cholesterol Level in Incubating Adrenal Homogenates—α-Ketoglutaric acid, acetic acid, phospho-enol pyruvic acid and glucose (in the presence and absence of monoiodoacetic acid) had no detectable effect upon the cholesterol concentration.

7. Effect of Incubation on Cholesterol Levels of Adrenal Homogenates—Incubation of adrenal homogenates at 37°C with DPN, ATP, cytochrome *c*, modified Krebs-Ringer buffer and phosphate buffer at pH 7.4 showed a definite decrease in cholesterol. When the gas phase above the incubation mixture was enriched with oxygen, this decrease ranged from 25 to 60 per cent.

8. Production of a New Steroid During Incubation—Concurrent with the cholesterol decrease, incubation apparently resulted in the appearance of another steroid in detectable quantities. From preliminary information two possibilities are Compound E and 17-hydroxycorticosterone.

Summary

1. The possibility of interconversion of cholesterol and other steroids in adrenal homogenates has been investigated.

2. The contribution to cholesterol synthesis of the addition of various "two-carbon fragment" producing substrates has been studied.

3. A procedure for the extraction, separation and determination of cholesterol in adrenal homogenates has been developed.

4. This method is used in conjunction with the Zaffaroni method for corticosteroid separation and with the Haines "Ultra-Violet Scanner" for steroid detection.

5. Fatty acid oxidase has been demonstrated in the adrenal gland but with a low activity.

6. A method for the separation and detection of acyl phosphates has been developed and applied to a study of the mechanism of α-ketoglutaric acid oxidation.

7. The addition of α-ketoglutaric acid, acetic acid, phospho-enol pyruvic acid, or glucose (with or without moniodoacetic acid) had no effect on the cholesterol content of incubating adrenal homogenates.

8. Incubation of adrenal homogenates results in a fall in the cholesterol concentration. This decrease is 25 to 60 per cent under on oxygen enriched atmosphere.

9. During incubation a ketosteroid is elaborated which is unidentified; two possibilities are Compound E and Compound F.

10. These studies support the proposition that cholesterol acts as a precursor for other steroids.

11. The methods used have been shown to be applicable to a further study of cholesterol metabolism.

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VITA

The author was born in New York City on May 17, 1922. He attended the public schools of that city and was graduated from DeWitt Clinton High School in June, 1938. He entered the College of the City of New York in Fall, 1938, and received the degree of Bachelor of Science in Chemistry from that institution in June, 1942. From June, 1942, until December, 1942, he worked as Junior Chemist at the General Foods Central Research Laboratories in Hoboken, New Jersey. In December, 1942, he entered the Army of the United States, serving in the American and European Theatres of Operation. He was honorably discharged in April, 1946, and returned to the General Foods Laboratories, where he worked until he entered the Graduate College of the University of Illinois in February, 1947. He held a teaching assistantship there in the Fall of 1947, and received the Master of Science degree from that university in February, 1948. From October, 1948, until June, 1950, he held an Upjohn Company research assistantship. He is a member of Sigma Xi, Phi Sigma and Gamma Alpha.